THE EFFECT OF Ca^{2+} ON OXYGEN EVOLUTION IN MEMBRANE PREPARATIONS FROM *ANACYSTIS NIDULANS*

Jerry J. BRAND

Department of Botany, The University of Texas, Austin, TX 78712, USA

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1. Introduction

Divalent cations are known to be important for proper structure and function of photosynthetic membranes. In most cases Mg^{2+} appears to be the physiological ion of importance although ATPase activity in certain photosynthetic coupling factor preparations is Ca^{2+}-dependent [1]. A direct role for Ca^{2+} in electron transport activities of isolated membranes from Cyanobacteria has been indicated [2,3].

Although *Anacystis nidulans* has been extensively used in studies of photosynthesis and other physiological problems, few investigations have utilized isolated photosynthetic membranes because of the difficulty in obtaining preparations which have retained their photochemical activities. Highly active preparations usually require incubation of cells with lysozyme under carefully controlled conditions, followed by separation of the membrane fraction from soluble components [4,5]. These preparations are often lengthy, and in our hands lead to variable activities from one preparation to the next. We report here a method for the rapid preparation of membrane fragments from *Anacystis* which give reproducibly high electron-transport activities when Ca^{2+} is present during cell breakage and during assays.

2. Materials and methods

Anacystis nidulans TX 20 was grown in continuously illuminated turbidostats as in [6]. Light intensity was maintained at 45 $\mu E \cdot m^{-2} \cdot s^{-1}$; temp. 39°C. Cells were maintained at ~7×10^8 cells/ml, equivalent to 1.6×10^{-2} mg chlorophyll/ml. Cell suspension (100 ml) was centrifuged (10 min, 10 000 × *g*) at 30°C. The pellet was resuspended in 20 ml complete resuspension medium (see table 1), or other media as indicated. All subsequent preparation steps were performed at 0–5°C. Resuspended cells were passed through a French pressure cell at 20 000 p.s.i. and at a 10 ml/min flow rate. This broken cell preparation was resuspended in a solution of 0.4 M sucrose and 0.05 M MES/tricine buffer (pH 7.3). The MES/tricine was prepared by prior mixing of 0.5 M stock solutions of tricine, NaOH pH 8.5 and MES NaOH (pH 5.5) to give the desired final pH. Chlorophyll concentration in the final suspension was 0.16 mg/ml. In some experiments broken cell preparations from the French pressure cell were first centrifuged (10 min, 60 000 × *g*) to pellet and remove unbroken cells. Very little material was pelleted in this step, and electron transport activities were identical regardless of whether this initial centrifugation was performed or not. Therefore, in all results reported here, only one centrifugation was performed, which recovered cell wall material as well as the photosynthetic and plasma membranes.

Oxygen evolution assays were performed with a Clark-type electrode, at 39°C and 2400 $\mu E \cdot m^{-2} \cdot s^{-1}$, supplied by a tungsten lamp. Chlorophyll concentration was measured spectrophotometrically [7].

3. Results

Membrane fragments from *Anacystis* gave high photochemical activities when cells were broken in

Table 1
Effect of cell resuspension medium components on photosystem II activity

Medium composition	% activity with complete medium
Complete[a]	100[b]
Minus sucrose	19
Minus Ca^{2+}	25
Minus Ficoll	100
Ca^{2+} replaced by Mg^{2+}[c]	44
pH 8.2	45

[a] Complete medium: 0.05 M MES/tricine buffer (pH 6.4); 0.36 M sucrose; 0.05 M $CaCl_2$; 0.0075 g/ml Ficoll
[b] O_2 evolution assays: 0.05 M MES/tricine buffer (pH 6.4); 1.33 mM potassium ferricyanide; 50 mM $CaCl_2$; membrane suspension containing 1.0 μg chlorophyll .ml^{-1}. 100% activity was 200 μmol O_2 .mg chlorophyll^{-1} .h^{-1}
[c] 0.05 M $MgCl_2$ substituted for $CaCl_2$

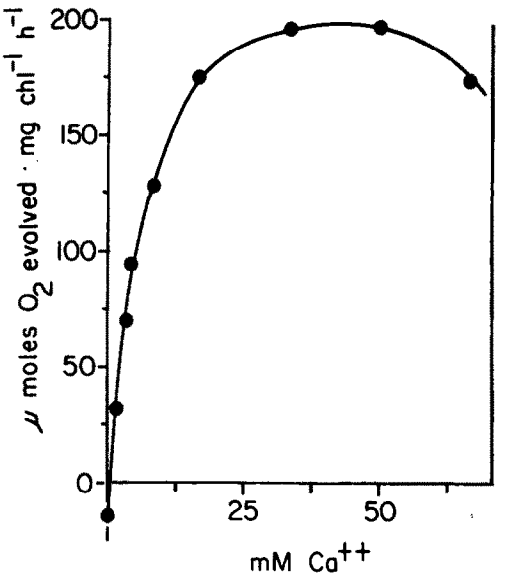

Fig.1. Effect of Ca^{2+} on photosynthetic O_2 evolution in membrane fragments from *Anacystis*. O_2 evolution assays contained components shown in table 1, using various Ca^{2+} concentrations.

the presence of an appropriate medium. Table 1 shows the relative importance of each component for photosystem II activity. An appropriate osmoticum was most important for subsequent activities. Divalent cations were also required for maximum activity, with Ca^{2+} serving this function much better than Mg^{2+}. Substitution of K^+ or Na^+ for Ca^{2+} in the French pressure cell medium did not stimulate activity beyond the rate with no added salt (data not shown). During French pressure cell disruption the suspension had to be maintained at pH < 7.3 for maximum activity in subsequent assays. Ficoll did not increase the rate of O_2 evolution, but activity did not decrease as rapidly with time following cell disruption when Ficoll was present during cell breakage.

The composition of the medium in which membranes were resuspended subsequent to pelleting was not as critical as in the cell breakage medium. Sucrose was necessary in the resuspension medium, but divalent cations and Ficoll had little effect on subsequent assays. Membrane fragments prepared as in section 2 using the complete medium of table 1 retained > 50% of their phycocyanin (determined by measuring the A_{620} before and after heating the membrane preparation to selectively destroy phycocyanin [8]).

Oxygen evolution assays required divalent cations for maximum activity; Ca^{2+} was by far the most effective ion tested. The effect of Ca^{2+} concentration on electron transport from H_2O to $K_3Fe(CN)_6$ is shown in fig.1. No O_2 was evolved in the absence of divalent cation; 30 mM Ca^{2+} saturated the reaction. As seen in table 2, some divalent cations partially substituted for Ca^{2+}, while others did not stimulate activity. Monovalent cations (K^+, Na^+ and NH_4^+ were tested) had no effect on the activity.

The Ca^{2+}-mediated rate of O_2 evolution was not further increased by any of the ions listed in table 2,

Table 2
Stimulation of photosystem II activity by various cations

Ion added[a]	% maximum activity
Ca^{2+}	100[b]
Mg^{2+}	41
Sr^{2+}	44
Mn^{2+}	36
Co^{2+}	0
K^+	0

[a] Each ion added as the chloride salt at 0.05 M
[b] O_2 evolution assays contained components shown in table 1, except cation composition as shown here. 100% activity was 200 μmol O_2 .mg chlorophyll^{-1} .h^{-1} in the presence of Ca^{2+}

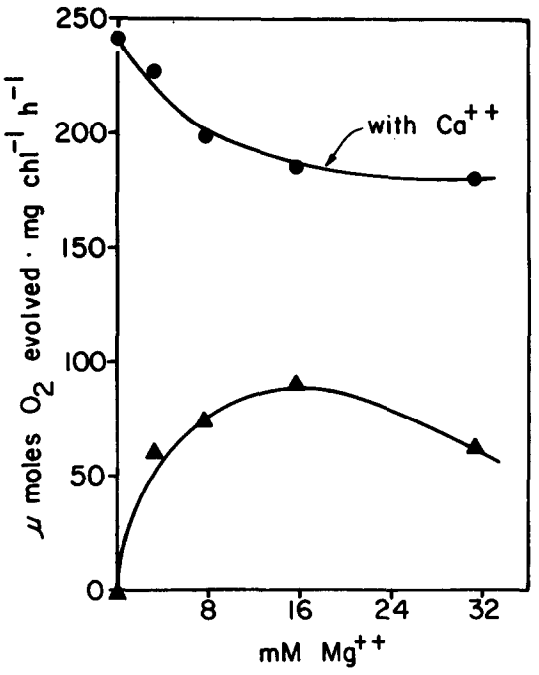

Fig.2. Additive effects of Ca^{2+} and Mg^{2+} on O_2 evolution in membrane fragments from *Anacystis*. Assays contained components shown in table 1, except for divalent cations. Triangles: assays contained no divalent cation except Mg^{2+} as $MgCl_2$ at concentrations indicated. Circles: assays contained 0.05 M $CaCl_2$ as well as $MgCl_2$ at concentrations indicated.

as shown in fig.2 for Mg^{2+}. In the absence of Ca^{2+}, Mg^{2+} is seen to facilitate the reaction somewhat, providing maximum activity at ~15 mM Mg^{2+}. Other divalent cations which substituted effectively for Ca^{2+} (table 2) also saturated at ~15 mM although Ca^{2+} saturated at ~30 mM. In the presence of saturating amount of Ca^{2+}, addition of Mg^{2+} did not further increase activity, but decreased it. This suggests that Mg^{2+} and Ca^{2+} may act at the same site, with Ca^{2+} acting most effectively.

The results reported here for O_2 evolution accompanying $K_3Fe(CN)_6$ reduction were essentially identical when benzoquinone served as electron acceptor, except the benzoquinone reaction gave some O_2 evolution in the absence of added divalent cation. Oxygen evolution in the presence of either ferricyanide or quinone was fully inhibited by 1×10^{-5} M DCMU, regardless of the divalent cation present. Photosystem I, measured by oxygen uptake accompanying electron transfer from Ascorbate/TMPD (*N,N,N',N'*-tetramethyl-*p*-phenylene diamine) to methyl viologen occurred at 236 μmol O_2 .mg chlorophyll^{-1} .h^{-1}. which was not further stimulated by any of the divalent cations listed in table 2.

4. Discussion

A Ca^{2+} requirement for O_2 evolution in the Cyanobacterium, *Phormidium luridum* was characterized [3] and similar Ca^{2+} effects in *Anacystis nidulans* and *Anabaena flosaquae* noted. Our preparations are similar to theirs in several respects. For example, maximum activity required the continued presence of an appropriate osmoticum following cell disruption; maximum activity required exposure of the isolated membranes to Ca^{2+}; the Ca^{2+}-stimulated activity was not further increased by a 'supernatant' factor when photosynthetic membranes were separated from other cytoplasmic components, in contrast to previous observations with Cyanobacteria [9]. However, our results differ from those reported [3,10] in several important ways. For example, in our preparations maximum activity required Ca^{2+} during cell breakage, and immediately prior to assays, but not during the intermediate period when membrane preparations were held near 0°C prior to assays; our rates routinely approached or exceeded 200 μmol O_2 .mg chlorophyll^{-1} .h^{-1}, ~20-times the rate seen previously with French pressure cell preparations of *Phormidium* and 8-times the previous rate with *Anacystis;* our preparations were fully sensitive to inhibition by low concentrations of DCMU. Our preparations were nearly as active in O_2 evolution accompanying benzoquinone reduction as in that accompanying ferricyanide reduction. Current investigation is aimed at elucidating the mechanism by which Ca^{2+} functions.

References

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